Review

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Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion

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Abstract

Astrocytes are housekeepers of the central nervous system (CNS) and are important for CNS development, homeostasis and defence. They communicate with neurones and other glial cells through the release of signalling molecules. Astrocytes secrete a wide array of classic neurotransmitters, neuromodulators and hormones, as well as metabolic, trophic and plastic factors, all of which contribute to the gliocrine system. The release of neuroactive substances from astrocytes occurs through several distinct pathways that include diffusion through plasmalemmal channels, translocation by multiple transporters and regulated exocytosis. As in other eukaryotic cells, exocytotic secretion from astrocytes involves divergent secretory organelles (synaptic-like microvesicles, dense-core vesicles, lysosomes, exosomes and ectosomes), which differ in size, origin, cargo, membrane composition, dynamics and functions. In this review, we summarize the features and functions of secretory organelles in astrocytes. We focus on the biogenesis and trafficking of secretory organelles and on the regulation of the exocytotic secretory system in the context of healthy and diseased astrocytes.

Keywords astrocytes; exocytosis; secretion; secretory vesicles; SNARE proteins
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See the Glossary for abbreviations used in this article.

Astrocytes, secretory cells of the CNS

The concept of astrocytes as secretory cells is almost as old as the discovery of these glial cells. The secretory potential of astrocytes became known only 15 years after Michael von Lenhossék coined the term "astrocyte" (von Lenhossék, 1895). In 1909, Hans Held observed, using the molybdenum haematoxylin stain, granular inclusions in neuroglial processes, which he interpreted as a sign of active secretion (Held, 1909). A year later, Jean Nageotte reported secretory granules in glial cells of the grey matter (i.e. astrocytes) using the Altmann method of fucsin labelling. Nageotte concluded that he was "able to present evidence of a robust and active secretion phenomenon in the protoplasm of these cells" (Nageotte, 1910). These granules, later called gliosomes by Alois Alzheimer (see (Glees, 1955) for historic narration), were often observed, and the hypothesis of astroglial secretion was also entertained by Wilder Penfield (Penfield, 1932). Of note, this early 20th century term should not be confused with the recent use of the name gliosomes for describing glial sub-cellular re-sealed particles (Nakamura *et al*, 1993) containing transmitter-laden vesicles (Stigliani *et al*, 2006). Be this as it may, both Nageotte and Penfield regarded astrocytes as true endocrine elements that release their secretions into the blood from their endfeet tightly associated with the brain vasculature. This endocrine role of astroglia has not been experimentally confirmed. However, research carried out in recent years has provided a remarkable body of evidence indicating that astrocytes secrete diverse substances that contribute to the regulation of CNS development and homeostasis, synaptogenesis and cognitive function. In that, astrocytes act as a part of a neuroglial secretory network, which, by analogy with the endocrine system, can be defined as the

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Glossary

Secretory vesicles	
SLMVs	synaptic-like microvesicles
DCVs	dense-core vesicles
ECVs	extracellular vesicles
MVBs	multivesicular bodies
Proteins mediating exocytosis	
SNARE	the soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptor. SNAREs are further sub-classified into R-SNAREs and Q-SNAREs. R-SNAREs are proteins contributing arginine (R) to the ionic layer of the ternary SNARE complex, whereas Q-SNAREs contribute glutamine (Q)
VAMP	vesicle-associated membrane protein Astrocytes express VAMP2, also known as synaptobrevin 2, VAMP3, also called cellubrevin, and tetanus toxin-insensitive VAMP (TI-VAMP), molecularly defined as VAMP7
SNAP-23	synaptosome-associated protein of 23 kDa
SNAP-25	synaptosome-associated protein of 25 kDa
SCAMP	secretory carrier membrane protein
Vesicular neurotransmitter transporters	
VNT	vesicular neurotransmitter transporter
VGLUTs	vesicular glutamate transporters, which belong to the SLC17 solute carrier family. All three known types, VGLUT1 (SLC17A7), VGLUT2 (SLC17A6) and VGLUT3 (SLC17A8), are expressed in astrocytes
VAChT (SLC18A3)	vesicular acetylcholine transporter
VMAT1 and 2 (SLC18A1 and SLC18A2, respectively)	vesicular monoamine transporters 1 and 2. VMAT 1 is also known as chromaffin granule amine transporter (CGAT)
VGAT (SLC32A1)	vesicular GABA transporter, also known as vesicular inhibitory amino acid transporter (VIAAT)
VNUT (SLC17A9)	vesicular nucleotide transporter
VEAT (SLC17A5)	vesicular excitatory amino acid transporter, also known as sialin
Other abbreviations	
8-Br-cAMP	8-bromo-adenosine 3′,5′-cyclic monophosphate, a membrane-permeable form of cAMP
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANP	atrial natriuretic peptide
ARRDC1	arrestin domain-containing protein 1, which interacts with the ESCRT
AQP4	aquaporin 4
BDNF	brain-derived neurotrophic factor
BFA	brefeldin A
CNS	central nervous system
DHPG	(R/S)-3,5-dihydroxyphenylglycine, an antagonist of mGluRs
EAAT	excitatory amino acid transporter
EGFP	enhanced green fluorescent protein
ESCRT	endosomal sorting complexes required for transport
FITC	fluorescein isothiocyanate
FM dyes	lipophilic styryl compounds used for studying vesicular recycling at the plasma membrane. Initially, they were synthesised by Fei Mao, hence FM
GFAP	glial fibrillary acidic protein
GluA	glutamate receptors, ionotropic AMPA type
GPCR	G protein-coupled receptor
HIV Tat	human immunodeficiency virus trans-activating proteins
IL-1β, IL-6, IL-18	interleukin-1β, interleukin-6, interleukin-18, respectively
IFN-γ	interferon-γ
LAMP1	lysosome-associated membrane glycoprotein 1, a lysosomal marker
MANT-ATP	(2′-(or-3′)-O-(N-methylanthraniloyl) adenosine 5′-triphosphate, a fluorescent analogue of ATP
MHC-II	major histocompatibility complex molecule class II
mGluRs	metabotropic glutamate receptors
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-D-aspartate
NPY	neuropeptide Y
NPR	natriuretic peptide receptor
PAR-4	protease-activated receptor 4
$PDGF_B$	platelet-derived growth factor subunit B
Rab proteins	a family of proteins, which are numerically denoted (e.g. Rab7, Rab11, Rab27 and Rab35) as members of the Ras superfamily of monomeric G proteins
ROS	reactive oxygen species
SLC	solute carrier
TIRF	total internal reflection fluorescence
TrkB receptor	tropomyosin-related kinase receptor
TSG101	tumour susceptibility gene 101, a component of ESCRT

gliocrine system of the CNS (Vardjan & Zorec, 2015). Other known cellular components of the gliocrine system are microglia and oligodendroglia, which all secrete numerous factors important for trophic support, homeostatic control and defence of the nervous tissue. It is highly likely that NG2 cells could be annexed to the gliocrine system, albeit experimental evidence on this account is lacking at present. Astroglia-derived secretory substances include (Table 1): (i) classical neurotransmitters, (ii) neurotransmitter precursors, (iii) neuromodulators, (iv) hormones and peptides, (v) eicosanoids, (vi) metabolic substrates, (vii) scavengers of ROS, (viii) growth factors, (ix) various factors that can be defined as "plastic" (e.g. factors that regulate synaptogenesis and synaptic connectivity) and, finally, (x) pathologically relevant molecules such as inflammatory factors. These different molecules are released by astrocytes through several pathways (Fig 1 and see Malarkey & Parpura, 2008 for details) represented by: (i) vesicle-based exocytosis (e.g. that of D-serine (Martineau *et al*, 2013) or glutamate (Montana *et al*, 2004); (ii) diffusion through plasmalemmal pores/channels (e.g. release of ATP and/or glutamate through anion channels, connexin hemichannels or dilated $P2X_7$ receptors, Cotrina *et al*, 1998; Suadicani *et al*, 2006) and (iii) extrusion through plasmalemmal transporters (e.g. the release of GABA via the reversed operation of GAT-3 transporters, Unichenko *et al*, 2012). Often, the same molecule can be released through different pathways, which affects the complexity/specificity of its action. The release of these molecules to the extracellular space, along with their subsequent transport by the convective glymphatic system (Thrane *et al*, 2014), occurs within various brain regions in different time

Table 1. Signalling molecules secreted by astrocytes.

Secreted substance	Secretion mechanism(s)	Function	Reference
(i) Neurotransmitters			
Glutamate	Exocytosis PM channels: Cx hemichannels; $P2X_7Rs$ Cystine-glutamate antiporter Reversed operation of glutamate transporters EAAT1/2—only in severe pathological conditions	Excitatory neurotransmitter acting on glutamate ionotropic (AMPA, KA and NMDA) and metabotropic receptors in neurones and neuroglia	Malarkey and Parpura (2008); Mazzanti *et al* (2001); Parpura *et al* (1994)
ATP	Exocytosis PM channels: Cx or Panx hemichannels, $P2X_7Rs$, other anion channels	Neurotransmitter acting on P2X receptors (excitatory action), P_2Y receptors (pleiotropic effects), and A_1 (inhibitory effects), A_{2A}, A_{2B} and A_3 (metabotropic effects) adenosine receptors in neurones and neuroglia	Queiroz *et al* (1997); Suadicani *et al* (2012); Vardjan *et al* (2014a)
GABA	Reversed operation of GABA transporters GAT1 (SLC6A1) and GAT3 (SLC6A11) PM channels: bestrophin1 (?)	Inhibitory neurotransmitter acting on neurones and on subpopulations of neuroglia	Heja *et al* (2009); Lee *et al* (2011); Unichenko *et al* (2013)
Glycine	Reversed operation of glycine transporters GlyT1 (SLC6A9)	Inhibitory neurotransmitter acting on neurones (mainly in the spinal cord) Co-agonist for NMDA receptors	Eulenburg and Gomeza (2010); Holopainen and Kontro (1989)
Neuropeptide Y	Exocytosis	Metabotropic neurotransmitter	Prada *et al* (2011)
(ii) Neurotransmitter precursors			
Glutamine	Sodium-coupled neutral amino acid transporters SNAT3/SLC38A3 and SNAT5/SLC38A5	Precursor for neuronal glutamate and GABA	Hertz (2013); McKenna (2007)
Pro-enkephalin	(?)	Precursor for enkephalins	Batter *et al* (1991)
(iii) Neuromodulators			
D-Serine	Exocytosis PM channels (?)	Co-agonist for NMDA receptors	Martineau *et al* (2014); Schell *et al* (1995)
Taurine	PM channels: Cx43, VRAC	Agonist for glycine and $GABA_A$ receptors	Kimelberg *et al* (1990)
L-aspartate	Exocytosis (?)	Positive modulator of NMDA receptors	
Kynurenic acid	(?)	Inhibitor of NMDA and acetylcholine receptors; aberrant production and synthesis can be associated with schizophrenia	Pershing *et al* (2015); Wu *et al* (2010)
(iv) Hormones and peptides			
Atrial natriuretic peptide (ANP)	Exocytosis	Local vasodilator	Krzan *et al* (2003)
Endothelin-3	(?)	Local vasoactive hormone	Ehrenreich *et al* (1991)
Sphingosine 1-phosphate	ATP-binding cassette transporter A1	Regulation of cell proliferation and immune response	Sato *et al* (2007)
Thyroid hormones thyroxine (T4) and triiodothyronine (T3)	L-type amino acid transporter 2 LAT2/SLC7A8	Astrocytes exclusively express type 2 deiodinase (D2) that converts T4 to T3. Astrocytes accumulate T4 by organic anion transporting polypeptide 1C1 (OATP1C1/SLCO1C1), convert it to T3, which is then released to brain parenchyma	Morte and Bernal (2014)
(v) Eicosanoids			
Arachidonic acid Prostanoids	Direct release from membranes	Multiple; including intercellular signalling and control of innate immunity	Murphy *et al* (1988); Xu *et al* (2003a,b)
(vi) Metabolic substrates			
Lactate	Membrane transporter Monocarboxylate Transporter MCT1/SLC16A1 Lactate channels	Possible energy substrate in neurones Possible activator of specific metabotropic receptors	Pellerin and Magistretti (2012); Sotelo-Hitschfeld *et al* (2015); Tang *et al* (2014)
Citrate	Transporters/Volume-regulated channels	Possible regulation of extracellular Ca^{2+} and Mg^{2+} (?)	

Table 1 (continued)

Secreted substance	Secretion mechanism(s)	Function	Reference
Glucose	Transporters GLUT1, GLUT2, GLUT3, GLUT4	Astrocytes may serve as a source for glucose being the only cells in the brain synthesizing glycogen	Prebil *et al* (2011); Muhic *et al* (2015)
(vii) ROS scavengers			
Glutathione	ATP-binding cassette sub-family B member 1 (ABCB1) Cx hemichannels	ROS buffering; astrocytes supply neurones with glutathione	Minich *et al* (2006); Rana and Dringen (2007)
Ascorbate	Na^+-dependent ascorbic acid transporter SVCT2/SLC23A2 Volume-sensitive anion channels (?)	ROS buffering; astrocytes regenerate extracellular ascorbate from its oxidized forms	Lane and Lawen (2013); Wilson *et al* (1991)
(viii) Growth factors			
Neurotrophins NGF NT-3 BDNF	Endo/exocytosis	Multiple trophic effects including regulation of neuronal survival, growth and regeneration	Ramamoorthy and Whim (2008); Toyomoto *et al* (2004); Stenovec *et al* (2015)
(ix) "Plastic" factors			
Thrombospondin-1	(?)	Regulation of synaptogenesis	Jayakumar *et al* (2014)
(x) Inflammatory factors			
IL-1	(?)	Control of neuroinflammatory response	Choi *et al* (2014)
IL-6	(?)	Control of neuroinflammatory response	Erta *et al* (2015)
C3a complement factor	Exocytosis, lysosomes (?)	Control of neuroinflammatory response	Lafon-Cazal *et al* (2003)

Mechanisms of release: vesicle-based regulated exocytosis (exocytosis), plasma membrane (PM) channels, transporters, extracellular vesicles (exosomes). Additional (to the Glossary) abbreviations: Cx, connexin; KA, kainate; Pnx, pannexin; VRAC, volume-regulated anion channels. ?, question mark indicates that the mechanism is still debatable or unknown.

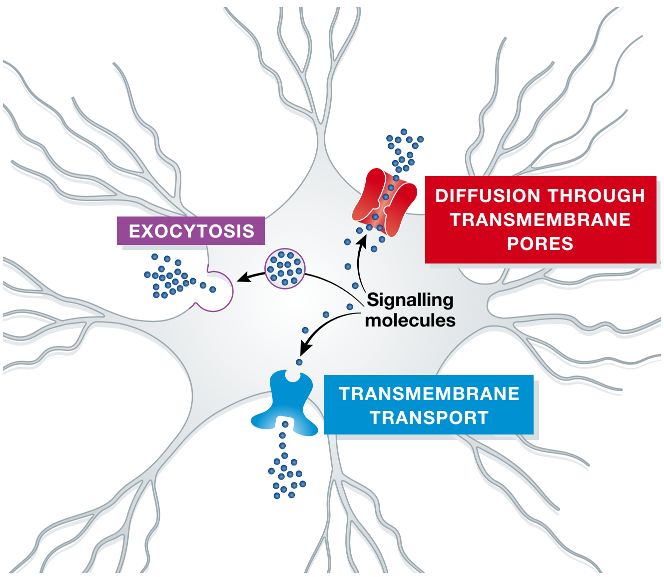

Figure 1. Multiple secretory pathways operating in astrocyte.

spans and with multiple functional consequences. In this review, we primarily focus on the exocytotic secretory pathway.

Exocytosis: multiple mechanisms

Exocytotic release, engaging various types of membrane-bound organelles laden with heterogeneous cargo, emerged early in evolution (Vardjan *et al*, 2010; Spang *et al*, 2015) and is present in the majority of eukaryotic cells. Fusion of organelles with the plasma membrane is key for intercellular signalling and for targeting various molecules (e.g. receptors or transporters) to the plasmalemma. Exocytosis is regulated by cytosolic free calcium ions and can occur either without stimulation (constitutive secretion) or in response to exogenous stimulation (regulated secretion, Kasai *et al*, 2012). In the brain, neurones are an exemplary model to study the exocytotic signalling pathway due to the spatially and temporally precise release of neurotransmitters at chemical synapses. Astrocytes are similarly capable of exocytosis, but this process is different in terms of spatial arrangements, kinetics and molecular mechanisms.

Vesicular release is supported by the evolutionary conserved family of SNARE proteins (Sollner *et al*, 1993). They are further divided into two categories, R-SNAREs and Q-SNAREs (Fasshauer *et al*, 1998; Jahn & Scheller, 2006). The former are associated with the vesicular membrane (also referred to as VAMPs), while the later are either integral plasma membrane proteins (e.g. syntaxins) or proteins associated with the plasma membrane (e.g. SNAP25 in neurones or SNAP23 in astrocytes). In the presence of supra-threshold cytosolic Ca^{2+} concentrations, R-SNARE and Q-SNARE proteins form the ternary SNARE complex by contributing their SNARE domains (one from each VAMP2 and syntaxin and two from SNAP23/25) to form a 4 α-helical bundle (SNAREpin). This bundle facilitates the fusion of vesicular and plasma membranes (Sutton *et al*, 1998; Weber *et al*, 1998). Kinetics of exocytosis is highly heterogeneous (Table 2). Fusion develops in < 1 ms in fast CNS synapses, whereas in endocrine or in kidney cells exocytosis proceeds over many hundreds of milliseconds or even seconds (Coorssen & Zorec, 2012; Kasai *et al*, 2012; Neher, 2012). Time course of

Table 2. Comparison of maximal rates of regulated exocytosis in different secretory cell types recorded as an increase in whole-cell membrane capacitance evoked by flash photolysis-induced elevations in cytosolic [Ca^{2+}].

Cell type	Max rate in regulated exocytosis	References
Endocrine cells		
Endocrine pituitary melanotrophes	25 s^{-1} 44 s^{-1}	Rupnik *et al* (2000); Thomas *et al*, 1993)
Endocrine pancreatic β cells	70 s^{-1}	Barg *et al* (2001); Wan *et al* (2004)
Chromaffin cells	1,500 s^{-1}	Voets (2000)
Neurones		
Rod photoreceptors	300 s^{-1} 400 s^{-1}	Kreft *et al* (2003); Thoreson *et al* (2004)
Retinal bipolar neurones	3,000 s^{-1}	Heidelberger *et al* (1994)
Inhibitory basket cell	5,000 s^{-1}	Sakaba (2008)
Calyx of Held neurones	6,000 s^{-1}	Bollmann *et al* (2000); Schneggenburger and Neher (2000)
Neuroglia		
Astrocytes	0.1–2 s^{-1}	Kreft *et al* (2004)

See also (Neher, 2012).

exocytotic release is determined by several factors. First, it is the sensitivity of secretory apparatus to $[Ca^{2+}]_i$, which is heterogeneous in different cell types. Second, the spatiotemporal progression of local $[Ca^{2+}]_i$ signals differs markedly between cells. For instance, in synaptic terminals excitation–secretion coupling is exceedingly fast due to the organisation of Ca^{2+} nanodomains that reflect a close proximity of the Ca^{2+} source and exocytotic machinery (Eggermann *et al*, 2012). Finally, slow regulated exocytosis may also evince a distinct vesicle nanoarchitecture (e.g. arrangement and density of R-SNAREs, see Fig 2) and the heterogeneity of Q-SNAREs (Takamori *et al*, 2006; Singh *et al*, 2014). Multiple mechanisms controlling exocytosis may coexist within the confinement of a single cell resulting in complex kinetics of secretion (Rupnik *et al*, 2000).

Diversity of astroglial secretory organelles

Eukaryotic cells produce different types of membranous secretory organelles that are classified as intracellular or extracellular. Intracellular vesicles are represented by transport vesicles, lysosomes and various types of secretory vesicles, whereas extracellular vesicles are ectosomes, exosomes, microvesicles (microparticles), membrane particles and apoptotic vesicles (van der Pol *et al*, 2012; Cocucci & Meldolesi, 2015). Intracellular vesicles are cellular organelles that may completely fuse with cellular membranes, whereas extracellular vesicles are membranous compartments released into the surrounding environment. Generally, vesicles undergoing constitutive or regulated exocytosis derive either from the trans-Golgi network or from early or recycling endosomes, although multivesicular bodies and lysosomes have been reported to undergo exocytosis under certain conditions.

Several secretory organelles undergo regulated exocytosis in astrocytes (Fig 3). These include clear electron lucent SLMVs that morphologically resemble synaptic vesicles (Bezzi *et al*, 2004; Crippa *et al*, 2006; Jourdain *et al*, 2007; Bergersen & Gundersen, 2009; Martineau *et al*, 2013), DCVs (Calegari *et al*, 1999; Parpura & Zorec, 2010) and secretory lysosomes (Zhang *et al*, 2007; Li *et al*, 2008; Verderio *et al*, 2012). All these organelles can store and release low (amino acids) and/or high (peptides and proteins) molecular weight chemical transmitters (Parpura & Zorec, 2010; Gucek *et al*, 2012; Vardjan & Zorec, 2015). Secretory vesicles can also act as recycling vesicles that take up extracellular molecules (e.g. by endocytosis) and promote their subsequent release (Vardjan *et al*, 2014b). This function may be essential for defining the composition of the cerebrospinal fluid and for the function of the glymphatic system (Thrane *et al*, 2014).

Synaptic-like microvesicles carry amino acids

Astroglial SLMVs typically have a diameter of 30–100 nm and appear in pairs/groups of 2–15 vesicles (Bezzi *et al*, 2004; Jourdain *et al*, 2007; Bergersen *et al*, 2012; Martineau *et al*, 2013). They are much less numerous compared to synaptic vesicles in nerve terminals where these organelles exist in groups of hundreds to thousands. Larger SLMVs (diameter of 1–3 μm) have also been identified in astrocytes in hippocampal slices. These vesicles may be generated by intracellular fusion of smaller vesicles and/or other organelles in response to a sustained increase in $[Ca^{2+}]_i$ or mechanical stimulation (Kang *et al*, 2013), but it is not clear whether they contribute to physiological secretion.

Concentrating neurotransmitters into vesicles is accomplished by vesicular neurotransmitter transporters or VNTs, which differ from the transporters at the plasma membrane with respect to energy coupling, substrate specificity and affinity. Six types of VNTs have been identified so far, including transporters for glutamate (VGLUT1-3), acetylcholine (vAChT), monoamines (VMAT1-2), GABA/glycine (VIAAT, also named VGAT), and more recently transporters for ATP (VNUT) and, possibly, for aspartate (sialin/VEAT) (Chaudhry *et al*, 2008; Sawada *et al*, 2008; Blakely & Edwards, 2012). Accumulation of D-serine in SLMVs is mediated by vesicular D-serine transporter, VSerT (Martineau *et al*, 2013), although its molecular identity remains elusive. The VNTs are essential molecular components of chemical transmission and the fingerprint of regulated exocytosis. Some VNTs such as VGLUT1-3 have been identified in cultured astrocytes (Fremeau *et al*, 2002; Bezzi *et al*, 2004; Kreft *et al*, 2004; Crippa *et al*, 2006; Montana *et al*, 2006). Analyses of astrocytes *in situ* using gene chip microarray, single-cell RT–PCR and immunostainings (Bezzi *et al*, 2004; Li *et al*, 2013; Sahlender *et al*, 2014) have produced variable results and in some cases have challenged the presence of VNTs and thus the concept of astroglial exocytosis. Nonetheless, immunogold electron microscopy, confocal microscopy and single-cell RT–PCR have shown that sub-populations of astrocytes in the brain express VGLUT1 (Bezzi *et al*, 2004; Bergersen *et al*, 2012; Ormel *et al*, 2012), VGLUT2 (Bezzi *et al*, 2004) and VGLUT3 (Ormel *et al*, 2012).

In astrocytes, SLMVs primarily store glutamate and D-serine, an agonist of glycine regulatory site of NMDA receptor (Martineau *et al*, 2008, 2013; Bergersen *et al*, 2012). In cultured astrocytes, SLMVs co-localise with D-serine (Mothet *et al*, 2005; Martineau *et al*, 2013) and VGLUTs, suggesting that glutamate and D-serine may reside in

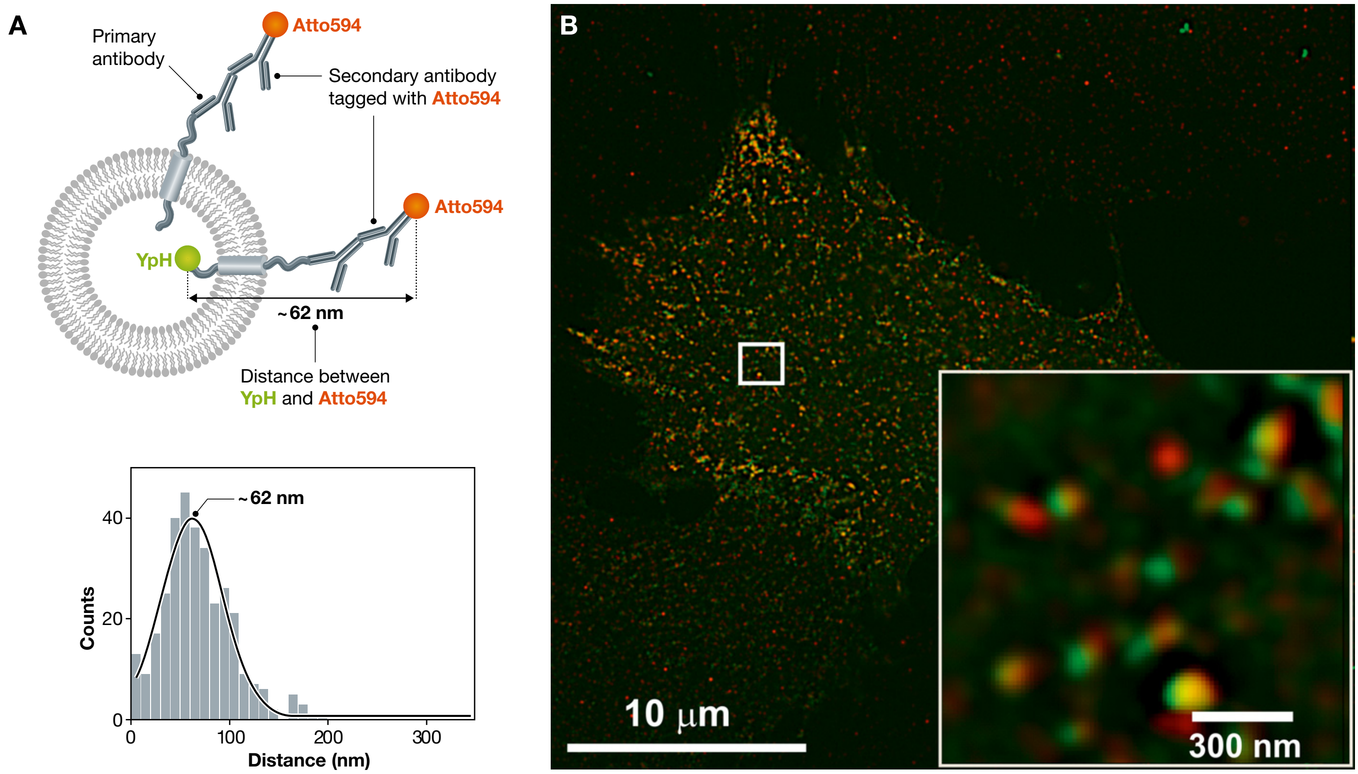

Figure 2. Arrangement of VAMP2 on vesicles in astrocytes.
(A) The diagram represents an astrocytic vesicle with the architecture of VAMP2 across the vesicle membrane. VAMP2 is appended at its luminal C-terminus with yellow phluorin (YpH, shown in green) and can be immunolabelled at its N-terminus (cytoplasmic site) using primary and secondary antibodies, the latter tagged with Atto594, a red fluorophore. The graph (below) shows the measurements of distance between the two fluorophores obtained from an astrocyte shown in (B), indicating the length of VAMP2 in astrocytes. (B) Structured illumination microscopy (SIM) micrograph of an astrocyte expressing VAMP2 marked fluorescently at luminal and cytoplasmic sites as schematized in (A). There is an incomplete co-localisation, that is separation, between the red and green puncta, disclosed as the distance in (A); co-localisation of YpH and Atto594 is disclosed in yellow. An area (box) of an astrocyte is shown in the inset (bottom right). Scale bar: 10 μm (300 nm in inset). (Modified with permission from Singh *et al*, 2014).

the same secretory organelle (Bezzi *et al*, 2004; Ormel *et al*, 2012). This contrasts the *in situ* evidence showing that glutamate and D-serine are stored in distinct SLMVs within the same astrocyte (Bergersen *et al*, 2012). Direct comparison of astroglial SLMVs (Crippa *et al*, 2006; Martineau *et al*, 2013) and neuronal synaptic vesicles shows that astrocytic vesicles in bulk contain D-serine and glutamate, whereas neuronal synaptic vesicles in bulk contain glutamate, glycine and GABA but are devoid of D-serine (Martineau *et al*, 2013; Sild & Van Horn, 2013). Astrocytes from various brain regions, including the hippocampus and cortex, and Bergmann glial cells in the cerebellum contain SLMVs (Bergersen *et al*, 2012; Ormel *et al*, 2012). These vesicles are present in perisynaptic processes as well as in somata (Bezzi *et al*, 2004; Montana *et al*, 2004; Ormel *et al*, 2012). The release of both glutamate and D-serine from astrocytes is Ca^{2+}-dependent and is blocked by tetanus toxin that cleaves astrocytic R-SNAREs VAMP2 and VAMP3 (Bezzi *et al*, 2004; Mothet *et al*, 2005; Martineau *et al*, 2008, 2014; Henneberger *et al*, 2010; Parpura & Zorec, 2010; Kang *et al*, 2013; Shigetomi *et al*, 2013).

Dense-core vesicles carry peptides

The DCVs are the main component for the storage and release of neuropeptides and hormones from neuroendocrine cells (Burgoyne & Morgan, 2003) and neurones (Klyachko & Jackson, 2002). These vesicles also contain ATP, which is likely accumulated into DCVs via VNUTs, albeit the presence of this transporter on these organelles has not yet been reported. The ultrastructure characteristics of astroglial DCVs are similar to those of neuroendocrine cells and neurones, although their core seems not as dense as in neuroendocrine cells (Potokar *et al*, 2008). The actual fraction of DCVs in astrocytes is quite small; for example, VAMP2-positive DCVs represent only 2% of the total number of vesicles examined (i.e. clear and dense-core vesicles, Crippa *et al*, 2006). Astroglial DCVs are generally larger than SLMVs, being ~100–600 nm in diameter (Calegari *et al*, 1999; Hur *et al*, 2010; Prada *et al*, 2011), albeit ANP-storing vesicles can have diameters as small as 50 nm (Potokar *et al*, 2008). DCVs from cultured astrocytes contain the secretory proteins secretogranins II (Calegari *et al*, 1999; Paco *et al*, 2009; Prada *et al*, 2011) and III (Paco *et al*, 2010), chromogranins (Hur *et al*, 2010), ANP (Kreft *et al*, 2004; Paco *et al*, 2009), neuropeptide Y (Ramamoorthy & Whim, 2008; Prada *et al*, 2011) and ATP (Coco *et al*, 2003; Pangrsic *et al*, 2007). The DCVs containing secretogranins were also identified in astrocytes from human brain samples (Hur *et al*, 2010), confirming the existence of DCVs *in situ*.

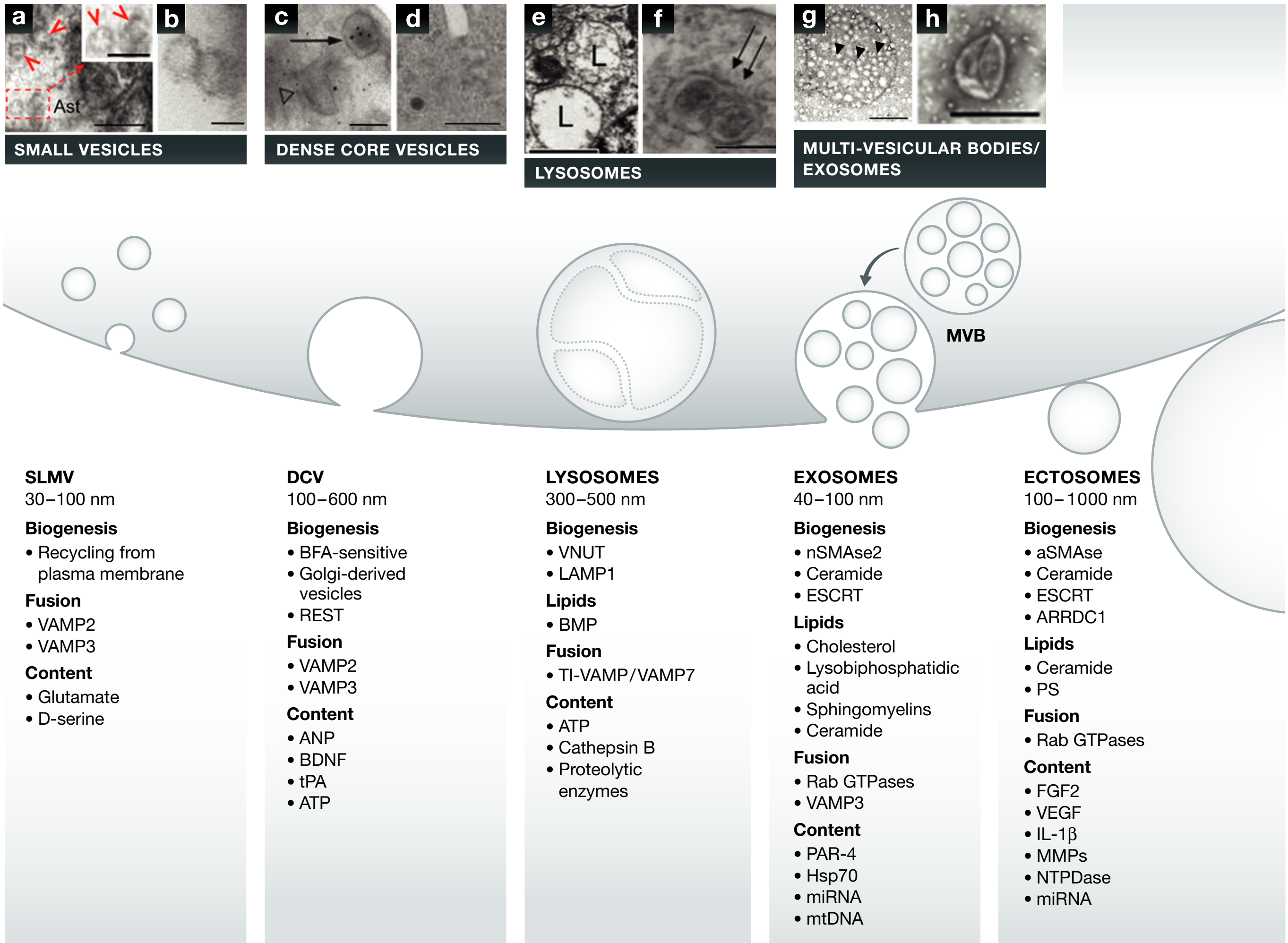

Figure 3. Diversity of astroglial vesicles.

Bottom panel shows schematic overview of astroglial secretory organelles. Top panel demonstrates ultrastructure of astroglial secretory organelles. (a) Electron micrograph of small clear vesicles (red arrowheads) in an astrocyte (Ast) from the rat hippocampus *in situ*. Scale bars: 100 nm (50 nm in inset). Modified with permission from Bergersen *et al* (2012). (b) Small clear vesicles from cultured astrocytes. Courtesy of Dr. Michela Matteoli. Scale bar: 50 nm. (c) Electron micrograph of a dense-core vesicle in cultured astrocytes stained by immunogold for secretogranin II. Modified with permission from Calegari *et al* (1999). Arrow shows DCV stained for secretogranin II. The open arrowhead points to the intermediate filament immunolabelled for GFAP. Scale bar: 100 nm. (d) Electron micrograph showing a dense-core vesicle in cultured astrocytes. Scale bar: 500 nm. Modified with permission from Prada *et al* (2011). (e) Electron micrograph of lysosomes (L) in astrocytes. Scale bar: 1 μm. ©2012 by National Academy of Sciences. Modified with permission from Di Malta *et al* (2012). (f) Electron microscopy images of an ADF glioma cell. Arrows point to multilamellar organelles. Scale bar: 150 nm. Modified with permission from Verderio *et al* (2012). (g) Electron micrograph showing a multivesicular body-like structure from a rat cultured astrocyte. Arrowheads indicate vesicles. Scale bars: 200 μm. Modified with permission from Brignone *et al* (2015). (h) Electron micrograph (negative staining) showing an exosome secreted by cultured astrocytes following stimulation with 100 mM BzATP, a P2X agonist, for 20 min. Scale bar: 300 nm. Modified with permission from Bianco *et al* (2009). Abbreviations: aSMase, acid sphingomyelinase; BMP, bis(monoacylglycero)phosphate; FGF2, fibroblast growth factor 2; Hsp70, 70 kilodalton heat shock protein; miRNA, microRNA; MMPs, matrix metalloproteinases; mtRNA, mitochondrial RNA; nSMase2, neutral sphingomyelinase 2; NTPDase, nucleoside triphosphate diphosphohydrolases; PS, phosphatidylserine; REST, RE-1–silencing transcription factor; tPA, tissue plasminogen activator; VGEF, vascular endothelial growth factor.

Secretory lysosomes in astrocytes

In cultured astrocytes, secretory lysosomes contribute to the storage and Ca^{2+}-dependent exocytotic release of ATP (Jaiswal *et al*, 2007; Zhang *et al*, 2007; Li *et al*, 2008). Diameters of secretory lysosomes are between 300 and 500 nm, and they coexist with SLMVs within the same astrocyte (Liu *et al*, 2011) and can be labelled with dextrans (Jaiswal *et al*, 2002; Vardjan *et al*, 2012), FM dyes and MANT-ATP (Zhang *et al*, 2007). These organelles are seemingly devoid of VGLUTs and VAMP2 (Zhang *et al*, 2007; Liu *et al*, 2011), while expressing lysosomal-specific markers such as cathepsin D, LAMP1 (Zhang *et al*, 2007; Martineau *et al*, 2008), ras-related protein Rab7, and VAMP7 (Chaineau *et al*, 2009). Secretory lysosomes also express VNUT (Sawada *et al*, 2008) that is needed for the accumulation of ATP (Oya *et al*, 2013). Exocytosis of lysosomes in astrocytes relies mainly on tetanus toxin-insensitive VAMP7, allowing for the release of both ATP and cathepsin B. Downregulation of VAMP7 expression inhibits the fusion of ATP-storing vesicles and ATP-mediated intercellular Ca^{2+} wave propagation (Verderio *et al*, 2012), a form of long-range communication in the astroglial network (Cornell-Bell *et al*, 1990). Fusion of secretory lysosomes is triggered by slow and locally restricted Ca^{2+} elevations (Li *et al*, 2008), which are distinct from Ca^{2+} spikes that are linked to SLMV fusion (Verderio *et al*, 2012). Similarly to other cells, secretory lysosomes in astrocytes are likely to play a role in membrane repair (Andrews & Chakrabarti, 2005).

Extracellular vesicles

Extracellular vesicles are broadly divided into exosomes and ectosomes. ECVs are typically loaded with a wide spectrum of bioactive substances including cytokines, signalling proteins, mRNA and microRNA (Mause & Weber, 2010). Exosomes are vesicles of 40–100 nm in diameter, produced through the formation of MVBs and their subsequent fusion with the plasma membrane (Mathivanan *et al*, 2010). Ectosomes, on the other hand, range from 100 to more than 1,000 nm in diameter and are formed and released by shedding off the plasma membrane.

The formation of exosomes follows the typical endocytic route, where transmembrane proteins are endocytosed and trafficked to early endosomes and subsequently to late endosomes. Intraluminal vesicles are generated by neutral sphingomyelinase 2 and ceramide-dependent process (Trajkovic *et al*, 2008) that also requires the ESCRT to generate MVBs (van Niel *et al*, 2006). Fusion of MVBs and release of exosomes involve Rab11, Rab27 and Rab35 (Vanlandingham & Ceresa, 2009; Hsu *et al*, 2010; Ostrowski *et al*, 2010; Baietti *et al*, 2012). During differentiation, MVBs become enriched in lipids such as cholesterol, lysobisphosphatidic acid and sphingomyelins containing ceramide (Kobayashi *et al*, 1998; Chevallier *et al*, 2008).

Ectosomes form by direct budding off the plasma membrane (Thery *et al*, 2009). Similarly to exosomes, ceramide is required for ectosome release (Bianco *et al*, 2009); ceramide together with ESCRT subunits participate in ectosome assembly and budding. During ectosome shedding from the plasma membrane, ARRDC1 interacts with ESCRT component TSG101 (Nabhan *et al*, 2012).

Astrocytes release both types of ECVs. Ectosome shedding from astrocytes occurs upon the activation of $P2X_7$ purinoceptors and involves the rapid activation of acid sphingomyelinase that moves to the plasma membrane outer leaflet. Sphingomyelinase alters membrane structure/fluidity leading to vesicle blebbing and shedding (Bianco *et al*, 2009). Diameters of ectosomes shed by cultured astrocytes from the 2-day-old rat cortex vary between 100 and 1,000 nm (Proia *et al*, 2008; Bianco *et al*, 2009). Some ECVs are even larger. For example, in cultured human foetal astrocytes spontaneous shedding of large (~4 μm diameter on average) ECVs has been detected. These large ECVs can contain mitochondria and lipid droplets and are decorated with β-1 integrin, a shedding marker (Falchi *et al*, 2013). The physiological relevance of this type of ECVs remains to be resolved; it cannot be excluded that they represent apoptotic bodies. Astrocyte-derived ectosomes carry numerous factors that regulate the activity of neighbouring cells including fibroblast growth factor 2 and vascular endothelial growth factor (Proia *et al*, 2008), IL-1β (Bianco *et al*, 2009), nucleoside triphosphate diphosphohydrolases (Ceruti *et al*, 2011) and matrix metalloproteinases (Sbai *et al*, 2010). Ectosomes also contain acid sphingomyelinase and high levels of phosphatidylserine on their membrane outer leaflet (Bianco *et al*, 2009). Finally, both exosomes and ectosomes contain nucleic acids, mainly microRNAs, small RNA regulators that have essential roles in different biological processes. Exosomes containing microRNAs can be utilized in communication between astrocytes and neurones. For instance, astrocytes treated with morphine and HIV Tat increase the expression of miR-29b that is released by exosomes; miR29b in turn is taken up by neurones where it downregulates the expression of $PDGF_B$ receptors (Hu *et al*, 2012). Of note, astrocyte-derived exosomes have been reported to contain mitochondrial DNA (Guescini *et al*, 2010). Whether ECVs also carry neurotransmitters is yet to be elucidated.

Exosomes are released from astrocytes in response to oxidative and heat stress (Taylor *et al*, 2007) and also in pathological conditions. Secretion of exosomes containing PAR4 and ceramide is increased in astrocytes surrounding amyloid plaques in a mouse model of familial Alzheimer's disease. These PAR4- and ceramide-enriched exosomes are subsequently taken up by astrocytes and induce apoptosis even in the absence of β-amyloid (Wang *et al*, 2012). Given the role of PAR4- and ceramide-containing exosomes in apoptotic processes, they are defined as "apoxosomes". Whether exosomes are discharged by astrocytes through a physiologically regulated process and whether exosomal release *in vivo* has a physiological function remains unclear. Owing to the lack of methods to specifically block exosome secretion without affecting secretion of other membrane vesicles, the resolution to these issues cannot be reached at the time being.

Molecular machinery of astroglial exocytosis

Twenty years ago, it was demonstrated that SNAREs are present in cultured astrocytes (Parpura *et al*, 1995). Subsequent studies have revealed that astrocytes express proteins characteristic for neuronal synaptic vesicles such as VAMP2 and proteins that are found in exocytotic trafficking vesicles of non-neuronal cells such as SCAMP and VAMP3 (Parpura *et al*, 1995; Maienschein *et al*, 1999; Wilhelm *et al*, 2004; Mothet *et al*, 2005; Crippa *et al*, 2006; Montana *et al*, 2006; Martineau *et al*, 2008). The lysosome-associated TI-VAMP/VAMP7 is expressed in astrocytes (Zhang *et al*, 2007; Martineau *et al*, 2008; Verderio *et al*, 2012) along with components of secretory machinery, Q-SNARE proteins SNAP23, syntaxins 1, 2, 3 and 4 (Hepp *et al*, 1999; Zhang *et al*, 2004b; Paco *et al*, 2009), SNARE-associated proteins such as synaptotagmin 4 (Zhang *et al*, 2004a) and isoforms of Munc18 (Paco *et al*, 2009). Cleavage of SNARE proteins with tetanus or botulinum neurotoxins (Verderio *et al*, 1999) reduce glutamate and D-serine and, to a lesser extent, ATP release in cultured astrocytes (Coco *et al*, 2003). Similarly, the treatment with tetanus toxin suppressed exocytosis measured by monitoring amperometric spikes (Chen *et al*, 2005) or by recording membrane capacitance (Kreft *et al*, 2004). The residual, toxin-insensitive component of Ca^{2+}-evoked exocytosis could be due to the contribution by other secretory organelles, such as lysosomes carrying toxin-insensitive VAMP7 (Verderio *et al*, 2012).

Several SNARE proteins, including VAMP2 (Wilhelm *et al*, 2004), VAMP3 (Bezzi *et al*, 2004; Zhang *et al*, 2004a; Jourdain *et al*, 2007; Bergersen & Gundersen, 2009; Schubert *et al*, 2011), VAMP7 (Verderio *et al*, 2012), SNAP23 and syntaxin 1 (Schubert *et al*, 2011), have been detected in astrocytes *in situ*. VAMP2 and 3 co-localise with VGLUT1 and 2 on SLMVs that store glutamate (Bezzi *et al*, 2004; Zhang *et al*, 2004a; Jourdain *et al*, 2007; Bergersen & Gundersen, 2009) and likely D-serine (Martineau *et al*, 2013). Several synaptotagmin isoforms including synaptotagmins 4, 5, 7 and 11 are also present in astrocytes (Zhang *et al*, 2004a; Mittelsteadt *et al*, 2009). However, typical neuronal SNARE-associated proteins, such as synaptotagmins 1 and 2, and synaptophysin (Wilhelm *et al*, 2004) have not been observed in astrocytes *in situ*.

Inactivation of VAMP2 and/or VAMP3 by tetanus neurotoxin abolished the release of glutamate (Jourdain *et al*, 2007; Perea & Araque, 2007) and, likely, D-serine in astrocytes in brain slices (Henneberger *et al*, 2010). A transgenic mouse model expressing a dominant negative (dn) SNARE (i.e. the cytosolic tail of VAMP2) in astrocytes (Pascual *et al*, 2005; Halassa *et al*, 2009) showed changes in behaviour, synaptic transmission and maturation of neurones (Pascual *et al*, 2005; Hines & Haydon, 2013; Nadjar *et al*, 2013; Turner *et al*, 2013; Lalo *et al*, 2014; Sultan *et al*, 2015), suggesting a role for astrocytic VAMP2-dependent exocytosis *in vivo*. Of note, VAMP2 cytosolic tail is supposed to compete with VAMP2 for binding to other components forming the ternary complexes, leading to the reduced number of complexes formed and hence inhibiting regulated exocytosis. Although these experiments provide strong support for a function of SNARE proteins in astroglial regulated exocytosis, there are indications that neurones might also express dnSNARE in the transgenic mice, thus raising the possibility that the impairment of neuronal, rather than astroglial, exocytosis may account for the phenotype observed (Fujita *et al*, 2014). The debate that ensued (Sloan & Barres, 2014) highlights technical matters, and particular aspects of astroglial glutamate secretion in the context of synaptic transmission, without questioning the general concept of exocytosis-mediated astroglial secretion. These technical dissensions nonetheless emphasize the need for refining the existing experimental strategies and developing new approaches directly attacking the various facets of astroglial secretion in physiological and pathophysiological contexts (Jahn *et al*, 2015).

Astroglial exocytosis is slow

Visualisation of fluorescently labelled VGLUT1/2-containing vesicles revealed that fusion events in isolated astrocytes occur within hundreds of milliseconds after the increase in cytosolic Ca^{2+} (Bezzi *et al*, 2004; Cali *et al*, 2008; Marchaland *et al*, 2008; Santello *et al*, 2011). Even slower kinetics of vesicular fusions has been reported by using synapto-pHluorin (spH), a fluorescently tagged-VAMP2, (Bowser & Khakh, 2007). Treatment of astrocytes with the Ca^{2+} ionophore ionomycin triggered exocytotic fusion of spH-labelled SLMVs within seconds (Liu *et al*, 2011). Similarly, the TIRF microscopy (Malarkey & Parpura, 2011) showed slow exocytotic bursts occurring within seconds after mechanical stimulation of astrocytes. Secretion of NPY from peptidergic vesicles occurred with a > 1-min delay after stimulation (Ramamoorthy & Whim, 2008; Prada *et al*, 2011). Exocytotic release from peptidergic vesicles in 8-Br-cAMP-matured astrocytes also began minutes after the stimulation (Paco *et al*, 2009). Similar observations have been made for secretory lysosomes, which labelled with FM dyes fused with the plasma membrane with an ~1-min delay after exposure of astrocytes to Ca^{2+} ionophores or ATP (Zhang *et al*, 2007; Li *et al*, 2008). Exocytotic fusion of quinacrine-loaded vesicles that express lysosomal VAMP7 occurred with a > 2-min delay after exposure to various stimuli including ionomycin, glutamate, ATP or UV-induced Ca^{2+} uncaging (Kreft *et al*, 2004; Pangrsic *et al*, 2007; Pryazhnikov & Khiroug, 2008). Likewise, EGFP-LAMP1- and FITC-dextran-labelled lysosomes underwent exocytotic fusion with a > 40-s delay after administration of ionomycin (Liu *et al*, 2011) or the group I mGluR agonist DHPG (Jaiswal *et al*, 2007).

Taken together, these imaging data indicate that in contrast to neurones, where the fusion occurs within < 0.5 ms after the Ca^{2+} entry into the cytosol (Neher, 2012; Sudhof, 2012), exocytotic release of various molecules from astrocytes is a much slower process, occurring with a substantial post-stimulus delay (Vardjan *et al*, 2015). Indeed, capacitance measurements on isolated astrocytes confirm that the kinetics of vesicle fusion is at least 2 orders of magnitude slower than in neurones (Fig 4 and Table 2; Kreft *et al*, 2003, 2004). Incidentally, inhibition of astroglial exocytosis (using astroglia targeted expression of dnSNARE or pharmacological tools) affects only slow electrical oscillations in the cortex (Fellin *et al*, 2009), while fast neuronal electrical activity seems to be unaffected by corrupted (using a mouse model rendering a reduction of VAMP1-3 expression in Müller cells, a specialized astroglia of the retina) gliotransmission (Slezak *et al*, 2012).

The somewhat lethargic kinetics of astroglial vesicular release likely reflects distinct organisation of the exocytotic machinery. First, electron microscopy studies (Bezzi *et al*, 2004; Jourdain *et al*, 2007; Bergersen *et al*, 2012) have shown that astrocytes lack structurally organised vesicle clusters typical of the active zone present in presynaptic terminals, which may make the stimulus–secretion coupling looser. In neurones, SNARE proteins are associated with vesicles clustered at active zones that are essentially release sites. This spatial localisation arguably is linked to the minimisation of the delay between the stimulus and the secretory output (Kasai *et al*, 2012). Second, the SNARE components and SNARE-associated proteins of the exocytotic apparatus are not identical in astrocytes and neurones, neither is the stability of SNARE complexes, nor are the numbers of SNARE molecules associated with a single vesicle.

VAMP isoforms with similar structural properties can participate in the formation of several different SNARE complexes (Wilhelm *et al*, 2004; Montana *et al*, 2009), which may affect the mechanism of vesicle fusion with the plasma membrane. In neuronal terminals, the ternary fusion complex forms between VAMP2, SNAP25 and syntaxin, whereas in astrocytes the ternary SNARE fusion complex assembles from VAMP2/3 or TI-VAMP, SNAP23 and syntaxin (Montana *et al*, 2009; Hamilton & Attwell, 2010). At a single molecule level, the presence of SNAP23A (as opposed to SNAP25B) in the ternary complex decreases the complex stability by half, arguably retarding the tethering/docking/fusion process (Fig 4B). Moreover, the density of R-SNAREs associated with a single vesicle in astrocytes is lesser than in neurones; in the latter, a single synaptic vesicle contains ~70 VAMP2 molecules (Takamori *et al*, 2006) vs. ~25 in a single astroglial vesicle (Singh *et al*, 2014). This paucity of VAMP2 would lead to the reduced density of ternary SNARE complexes, which would contribute to further retardation of docking and fusion process in astrocytes.

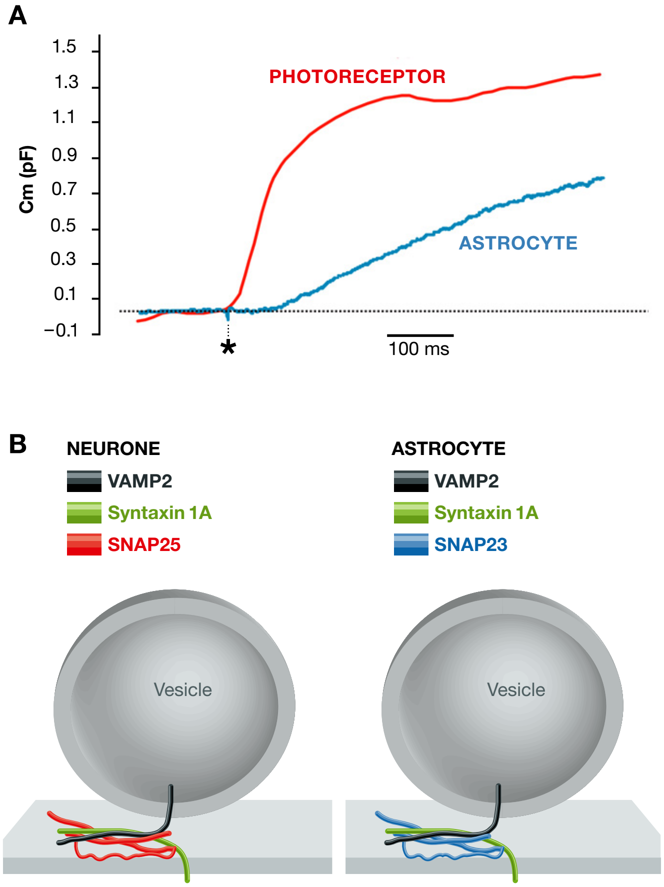

Figure 4. Slowness of astroglial exocytosis.
(A) Comparison of kinetics of neuronal and astroglial exocytosis. Time-dependent changes in membrane capacitance (Cm) recorded in a neuronal cell (trace in red, photoreceptor, modified from Kreft *et al*, 2003) and an astrocyte (trace in blue, modified from Kreft *et al*, 2004), elicited by a flash photolysis-induced increase in cytosolic Ca^{2+}. Note that the blue trace recorded in an astrocyte displays a significant delay between the stimulus (asterisk) and the response (trace components above the dotted line). (B) Neuronal vs. astrocytic SNAREs. Neurones and astrocytes alike express SNAREs VAMP2 and syntaxin 1; many astrocytes can also express VAMP3 in lieu of or in addition to VAMP2. Astrocytes express SNAP23, a homologue of neuronal SNAP25. At the plasma membrane, syntaxin 1A can form a binary *cis* complex with SNAP25B or SNAP23A, which then interacts with vesicular VAMP2 to form a ternary complex. A single ternary complex can tether the vesicle at the plasma membrane for a longer period of time (1.9 s vs. 0.8 s), when it contains SNAP25B rather than SNAP23A, respectively. Of note, truncated syntaxin 1, lacking the N-terminal Habc domain and the linker region to the SNARE domain, is shown for simplicity. Drawings are not to scale.

Trafficking of astroglial vesicles

Quantitative measurements of secretory vesicles mobility in astrocytes (Potokar *et al*, 2005, 2013b) revealed two types of vesicle mobility: (i) directional, when vesicles travel along tracks, such as cytoskeletal elements, including intermediate filaments, or (ii) non-directional, characterised by contorted vesicle trajectories, typical for the Brownian movement of particles. These experiments also unveiled a dichotomy of vesicular traffic: glutamatergic vesicles accelerate with an increase of cytosolic Ca^{2+} (Stenovec *et al*, 2007), whereas peptidergic vesicles and endolysosomes slow down (Potokar *et al*, 2008, 2010). Such stimulation-dependent vesicle mobility regulation has not been observed in neurones and may represent an adaptive mechanism for astrocytes to redistribute vesicles to the correct location.

Astroglial exocytosis in physiology and pathophysiology

Endo/exocytosis of BDNF

BDNF is a powerful regulator of neuronal plasticity (Poo, 2001). Its synthesis, which occurs in both neurones and astrocytes (Lu *et al*, 2005; Juric *et al*, 2008), yields two distinct forms: pro-BDNF (which binds to and acts through the pan-neurotrophin receptor p75) and mature BDNF that stimulates TrkB receptor. Neurones often release pro-BDNF, which undergoes maturation either extracellularly (by tissue plasminogen activator/plasmin) or in astroglia. The latter pathway has been demonstrated in hippocampal slices and involves endocytotic uptake of pro-BDNF by astrocytes, in response to a strong electrical stimulation of neurones, conversion of pro-BDNF into the mature form in astrocytes and subsequent VAMP2-mediated exocytotic release of mature BDNF from these glial cells (Bergami *et al*, 2008).

Secretion of peptides

Astrocytes also synthesize and secrete NPY, a peptide widely distributed throughout the mammalian nervous system (Barnea *et al*, 1998, 2001), where it acts as a neuroproliferative factor (Hansel *et al*, 2001; Geloso *et al*, 2015) and regulates the growth of vascular tissue (Zukowska-Grojec *et al*, 1993). Release of NPY is activated by an mGluR-linked increase in cytosolic Ca^{2+} and proceeds through exocytotic fusion of DCVs (Ramamoorthy & Whim, 2008). Several types of natriuretic peptides, including ANP, brain natriuretic peptide and C type natriuretic peptide (CNP), are present in the CNS (Potter *et al*, 2006). ANP in particular is present in neurones and astrocytes in various brain regions (McKenzie *et al*, 2001). Natriuretic peptides exert their actions by binding to natriuretic peptide receptors (NPRs). ANP binds preferentially to NPR-A, while brain natriuretic peptide and CNP bind to NPR-B receptors; all NPs bind with equal affinity to NPR-C (Lucas *et al*, 2000). NPR-A and NPR-B are plasmalemma-bound guanylyl cyclase receptors, which mediate intracellular signalling by increasing intracellular cGMP. NPR-C is a "clearance receptor" that removes peptides from the extracellular space, but does not itself possess guanylyl cyclase activity (Potter *et al*, 2006; Rose & Giles, 2008). In astrocytes, ANP is stored in vesicles and released into the extracellular space by regulated exocytosis (Krzan *et al*, 2003). The astroglial ANP content significantly increases after experimental brain infarction (Nogami *et al*, 2001), suggesting that this gliosignalling molecule may regulate the cerebral blood flow. ANP is also involved in the control of systemic salt intake as the loss of ANP receptors eliminates the inhibition of salt-seeking behaviour caused by a NaCl load (Blackburn *et al*, 1995).

Delivery of receptors, channels and transporters to the plasma membrane

Ionotropic glutamate receptors The VAMP2-positive vesicles of cultured astrocytes are immunopositive for the AMPA receptor subunits GluA2,3 and, to a lesser extent, for GluA1 (Crippa *et al*, 2006). This presence of GluA2,3 subunits on VAMP2-positive vesicles suggests a vesicle-mediated mode of AMPA receptor delivery to the astrocytic plasma membrane, as previously described in neurones (Passafaro *et al*, 2001).

Glutamate transporter EAAT2 Astrocytes play a key role in the uptake of glutamate released during synaptic transmission (Danbolt, 2001). Glutamate clearance is a function of Na^+-dependent excitatory amino acid transporters EAAT1 and EAAT2, which are predominantly expressed in astroglia (Zhou & Danbolt, 2013). The efficacy of the clearance directly depends on the density of transporters in the plasma membrane (Robinson, 2002; Huang & Bergles, 2004). The density of EAAT2 in astrocyte plasmalemma is controlled by exo-/endocytosis (Stenovec *et al*, 2008; Li *et al*, 2015). Aberrant trafficking of EAAT2-containing vesicles to the plasma membrane may compromise glutamate uptake and contribute to neuronal excitotoxicity such as seen in amyotrophic lateral sclerosis (Rossi, 2015).

G protein-coupled receptors (GPCRs) Astrocytes express several types of GPCRs such as cannabinoid receptor 1, CBR1 (Navarrete & Araque, 2008), chemokine receptor CXCR4 (Bezzi *et al*, 2001), mGluR5 (Kirischuk *et al*, 1999) and $P2Y_1$ purinoceptors (Domercq *et al*, 2006). The delivery of GPCRs to the plasmalemma may involve vesicular transport. CBR1 is mainly expressed in acidic intracellular organelles that co-localise with endocytic compartments. While trafficking of CBR1 has been studied using CBR1 fluorescent proteins chimeras, the mechanism by which it reaches the surface of astrocytes, whether by a constitutive recycling pathway or by a Ca^{2+}-dependent mechanism such as exocytosis, remains to be determined (Osborne *et al*, 2009).

Aquaporin 4 Astrocytes express aquaporin 4 (AQP4), a channel that is critical for brain water homeostasis (Nagelhus & Ottersen, 2013). Distribution of AQP4 in astrocytes is highly polarised being mainly confined to endfeet, and to a lesser extent, to perisynaptic processes (Nielsen *et al*, 1997; Nagelhus *et al*, 1998; Arcienega *et al*, 2010). Water transport mediated by AQP4 contributes to pathology and is important for astrocyte swelling and brain oedema formation/resolution *in vitro* (Yamamoto *et al*, 2001; Arima *et al*, 2003) and *in vivo* (Ke *et al*, 2001; Papadopoulos *et al*, 2004). Water transport through the cell membrane is regulated by the permeability properties of AQP4 (Gunnarson *et al*, 2008; Nicchia *et al*, 2011), the heterogeneity of AQP4 crystalline-like orthogonal arrays of particles (Hirt *et al*, 2011) and, as recently suggested, by trafficking of AQP4-containing vesicles to/from the plasma membrane (Potokar *et al*, 2013a). In unstimulated conditions, the mobility of vesicles containing AQP4 resembles the mobility of slow recycling and endosomal vesicles. This mobility of AQP4e isoform-laden vesicles correlated with changes in the AQP4 presence at the plasma membrane. Hypoosmotic stimulation, which induces astrocyte swelling, triggered a transient reduction in AQP4e isoform vesicle mobility mirrored by the transient increase in the AQP4 plasma membrane expression. These data indicate that the regulation of vesicle mobility is an important mechanism to alter the delivery/retraction ratio of AQP4 vesicles to/from the astroglial plasma membrane.

Control of sleep homeostat

An increase of adenosine levels in the extracellular space promotes sleepiness, while; adenosine receptor antagonists promote wakefulness (Basheer *et al*, 2004). It turns out that astrocytes are key for controlling adenosine levels and they do so via SNARE-dependent release of ATP that is converted to adenosine extracellularly by ecto-nucleotidases (Pascual *et al*, 2005). Thus, astroglial sourced adenosine is essential for the regulation of sleep homeostat and for responses to sleep deprivation (Halassa *et al*, 2009).

Cocaine addiction

Astrocytes also contribute to the extracellular level of glutamate in the nucleus accumbens core (NAcore) by a SNARE-dependent process. At behavioural level, cue-induced reinstatement of cocaine seeking in rats extinguished from cocaine was inhibited by glutamate release from astrocytes, which action was mediated via the group II mGluRs (Scofield *et al*, 2015). Of note, stimulation of inhibitory presynaptic mGluR2/3 receptors reduces synaptic glutamate release in the NAcore, preventing drug seeking. Cocaine addiction is also characterised by impaired NMDA receptor-dependent synaptic plasticity in the NAcore. It has been shown that cocaine-induced deficits in NMDAR-dependent long-term potentiation and depression result partially from reduced release of D-serine from astrocytes (Curcio *et al*, 2013). Administration of D-serine directly into the NAcore *in vivo* blocked behavioural sensitisation to cocaine. Accordingly, D-serine and glutamate could team up to regulate the cocaine sensitisation state.

Glial exocytosis in neuroinflammation: secretion of complement proteins

The complement system represents one of the most fundamental immune regulating cascade, defining various aspects of tissue defence (Holers, 2014). Complement proteins C3a and C1q are present in the CNS, where they regulate neurogenesis, neuronal survival and synaptic elimination (Stevens *et al*, 2007; Shinjyo *et al*, 2009). The C3a complement protein is produced and secreted from astroglia; this secretion is disrupted by brefeldin A, which interferes with anterograde transport from the endoplasmic reticulum to the Golgi apparatus, thus indicating the specific role for the secretory pathway (Lafon-Cazal *et al*, 2003). NF-κB signalling promotes secretion of C3a and excessive NF-κB activation may increase astroglial C3a release that in turn can contribute to neurodegeneration (Lian *et al*, 2015).

Glial exocytosis in neuroinflammation: antigen presentation

In neuropathology, astrocytes often become reactive, which leads to their morphological and biochemical remodelling; the reactivity is manifested by an increased expression of intermediate filaments (most notably GFAP and vimentin) (Burda & Sofroniew, 2014; Pekny *et al*, 2014; Sofroniew, 2015). Reactive reprogramming of astrocytes also affects vesicle delivery. Exposure of otherwise immunologically silent astrocytes to interferon-γ, a proinflammatory cytokine, initiates expression of MHC-II molecules and surface antigens causing astroglial cells to behave like nonprofessional antigen-presenting cells (Vardjan *et al*, 2012). It has been suggested that IFN-γ-activated astrocytes participate in antigen presentation and activation of CD4 helper T cells in immune-mediated disorders of the CNS including multiple sclerosis (Fontana *et al*, 1984; Soos *et al*,

1998) and experimental autoimmune encephalomyelitis (Shrikant & Benveniste, 1996).

The delivery of MHC-II molecules to the cell surface of antigen-presenting cells is mediated via a cytoskeletal network and requires the fusion of MHC-II-carrying late endolysosomes with the plasma membrane. Actin microfilaments (Barois *et al*, 1998), microtubules (Wubbolts *et al*, 1999; Vyas *et al*, 2007) and their motor proteins (Wubbolts *et al*, 1999; Vascotto *et al*, 2007) mediate trafficking of MHC-II compartments in antigen-presenting cells. Recently, the role of intermediate filaments (GFAP and vimentin) in MHC-II trafficking was investigated in IFN-γ-activated astrocytes (Vardjan *et al*, 2012). In IFN-γ-activated astrocytes, upregulation of intermediate filaments allows for a faster and therefore more efficient delivery of MHC-II molecules to the cell surface (Vardjan *et al*, 2012). Reduced mobility of late endolysosomes due to an increase in $[Ca^{2+}]_i$ may increase their probability of docking and fusion to the plasmalemma (Potokar *et al*, 2010), which, in astrocytes acting as antigen-presenting cells, may provide an additional regulatory mechanism that controls the delivery of MHC-II molecules to the cell surface (Vardjan *et al*, 2012). Besides IFN-γ, endogenous suppressors, including norepinephrine, regulate the expression of MHC-II molecules in astrocytes (Frohman *et al*, 1988; De Keyser *et al*, 2004). The effects of norepinephrine are mediated through the activation of G protein-coupled β-adrenergic receptors on astrocytes and the activation of the cAMP signalling pathway (Vardjan *et al*, 2014b). However, it is unclear how this pathway controls the vesicular delivery of MHC-II molecules to the plasma membrane. These regulatory mechanisms may enable antigen-presenting reactive astrocytes to respond rapidly and in a controlled manner during CNS inflammation. Incidentally, cultured astrocytes expressing mutated (M164V) presenilin 1 have impaired vesicular trafficking, which may be related to compromised defensive capabilities of astrocytes in the neurodegeneration context (Stenovec *et al*, 2016).

Glial exocytosis in neuroinflammation: release of cytokines with ECVs

Human astrocytes express a large number of cytokines (Choi *et al*, 2014). The mechanisms by which astrocytes secrete these cytokines are still to be defined. However, the release of pro-inflammatory cytokines, and in particular IL-1β, has been extensively characterised in microglia. Microglia extracellular vesicles express IL-1β, IL-6, inducible nitric oxide synthase and cyclooxygenase-2 (Bianco *et al*, 2009; Verderio *et al*, 2012). Microglial ectosomes contain the cytokine IL-1β (Bianco *et al*, 2005, 2009). Pro-IL-1β is incorporated into ectosomes together with pro-caspase-1, the enzyme responsible for IL-1β maturation, $P2X_7$ receptor (Bianco *et al*, 2005), and likely with other inflammosome components, as described in monocytes (Qu *et al*, 2007; Sarkar *et al*, 2009). As a consequence of the assembly of this multiprotein complex, mature IL-1 β (as well as IL-18) is released from ectosomes upon ATP stimulation. It is possible that proinflammatory cytokines from astrocytes follows a similar route, employing extracellular vesicles.

Concluding remarks

Astrocytes express a complex exocytotic machinery that is associated with several types of secretory vesicles involved in the secretion of a wide variety of neurotransmitters, neurotransmitter precursors, hormones, trophic and plastic factors, etc. Astroglial secretion contributes to the intrinsic CNS gliocrine network that provides for the regulation of multiple physiological and pathophysiological processes. Likely owing to the difference in secretory machinery, astroglial exocytosis is much slower that the neuronal counterpart. This fundamental difference reflects distinct physiological specialisation of astroglia as a key homeostatic component of the neural network.

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Conflict of interest

The authors declare that they have no conflict of interest.

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